Stroke

ORIGINAL CONTRIBUTIONS

Revacept, an Inhibitor of Platelet Adhesion in Symptomatic Carotid Stenosis: A Multicenter Randomized Phase II Trial

Timo Uphaus, MD; Toby Richards, MD; Christian Weimar, MD; Hermann Neugebauer, MD; Sven Poli, MD; Karin Weissenborn, MD; Christopher Imray, MD; Dominik Michalski, MD; Hisham Rashid, MD; Ian Loftus, MD; Christian Rummey, PhD; Martin Ritter, MD; Till-Karsten Hauser, MD; Götz Münch, MD; Klaus Gröschel, MD*; Holger Poppert, MD*

BACKGROUND: Patients with symptomatic internal carotid artery (ICA) stenosis are at high risk of recurrent ischemic stroke and require early interventional treatment and antiplatelet therapy. Increased bleeding rates might counterbalance the periprocedural efficacy of intensified platelet inhibition. We aim to investigate, whether Revacept, a competitive antagonist of glycoprotein VI, adjunct to standard antiplatelet therapy reduces the occurrence of ischemic lesions in patients with symptomatic ICA stenosis.

METHODS: International, multicenter (16 sites), 3-arm, randomized (1:1:1), double-blind, and placebo-controlled study with parallel groups, including patients with symptomatic ICA stenosis. A single infusion over 20 minutes of either placebo, 40 mg or 120 mg Revacept in addition to guideline-conform antiplatelet therapy was evaluated with regard to the exploratory efficacy end point: Number of new ischemic lesions on diffusion-weighted magnetic resonance imaging after treatment initiation. Main clinical outcome was the combined safety and efficacy end point including any stroke or death, transient ischemic attack, myocardial infarction, coronary intervention, and bleeding complications during follow-up.

RESULTS: Out of 160 randomized patients, 158 patients (68±10.1 years, 24% female) received study medication (51 patients placebo, 54 patients 40 mg Revacept and 53 patients 120 mg Revacept) and were followed for 11.2±2.3 months. A total of 1.16 (95% CI, 0.88–1.53)/1.05 (95% CI, 0.78–1.42; *P*=0.629)/0.63 (95% CI, 0.43–0.93) new diffusion-weighted magnetic resonance imaging lesions per patient were detected in the placebo/40 mg/120 mg Revacept groups, without statistical evidence of a difference. A reduction of the combined safety and efficacy end point during the study period was observed in patients who received 120 mg (HR, 0.46 [95% CI, 0.21–0.99]; *P*=0.047), but not 40 mg Revacept compared with placebo (HR, 0.72 [95% CI, 0.37–1.42]; *P*=0.343).

CONCLUSIONS: Revacept 120 mg reduced the combined safety and efficacy end point in patients with symptomatic ICA stenosis.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique Identifier: NCT01645306.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: carotid artery, internal ■ glycoprotein ■ ischemic attack, transient ■ magnetic resonance imaging ■ revacept

Correspondence to: Klaus Gröschel, MD, University Medical Center of the Johannes Gutenberg University Mainz, Department of Neurology, Langenbeckstr. 1, 55131 Mainz, Germany. Email klaus.groeschel@unimedizin-mainz.de
*K. Gröschel and H. Poppert contributed equally.
This manuscript was sent to Ajay K. Wakhloo, Guest Editor, for review by expert referees, editorial decision, and final disposition.
Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.121.037006.
For Sources of Funding and Disclosures, see page XXX.

Stroke is available at www.ahajournals.org/journal/str

ORIGINAL CONTRIBUTIONS

Nonstandard Abbreviations and Acronyms

ASA	acetylsalicylic acid
BMT	best medical treatment
CARESS	Clopidogrel and Aspirin for Reduction of Emboli in Asymptomatic Carotid Stenosis
CAS	carotid angioplasty and stenting
CEA	carotid endarterectomy
DWI	diffusion-weighted magnetic resonance imaging
ECST	European Carotid Surgery Trial
Fc	fragment crystallizable
GPIb	glycoprotein Ib
GPVI	glycoprotein VI
ICA	internal carotid artery
ISAR-PLASTER	Intracoronary Stenting and Antithrombotic Regimen: Lesion Platelet Adhesion as Selective Target of Endovenous Revacept
MCA	middle cerebral artery
MES	microembolic signals
MRI	magnetic resonance imaging
NASCET	North American Symptomatic Carotid Endarterectomy Trial
NIHSS	National Institutes of Health Stroke Scale
RE-LY	randomized evaluation of long-term anticoagulant therapy
TCD	transcranial doppler sonography
TIA	transient ischemic attack

Patients with recent cerebral ischemic infarction because of internal carotid artery (ICA) stenosis are at high risk of recurrent cerebrovascular events.[1,2] The underlying pathophysiological process for this early stroke reoccurrence is thought to be ongoing embolization because of activation of circulating platelets by exposed collaged at the site of ruptured/vulnerable plaque.[3] Identification of such "hot plaques," which carry the highest risk of recurrent ischemic stroke, has been realized with transcranial Doppler sonography (TCD) and detection of microembolic signals (MES),[4,5] which are thought to represent active distal embolization from the vulnerable plaque. However, diffusion-weighted magnetic resonance imaging (DWI) is regarded the most sensitive method for in-vivo assessment of the resulting brain damage including smallest lesions.[6]

Early initiation of dual antiplatelet treatment aims at reducing active embolizations, and has been shown to diminish MES,[5] new ischemic lesions and early stroke reoccurrence,[7] but an excess of bleeding complications, particularly in the elderly, may counterbalance this risk reduction.[8]

Revacept, a protein made out of a fragment crystallizable region fused to the GPVI (glycoprotein VI) receptor, which is an endogenous platelet collagen receptor, binds to the exposed collagen of the unstable carotid plaque and acts as a physical barrier, reducing platelet activation and subsequent platelet binding and aggregation on the carotid plaque.[9,10] Due to Revacepts' half-life of around 7 to 14 days, it might be a site-specific strategy to face the high risk of early recurrent cerebrovascular events after symptom onset of symptomatic ICA stenosis, as well as bridge the time-interval between symptom onset and revascularization procedure, without compromising systemic platelet function.

We hypothesized that the use of the novel inhibitor Revacept in patients with symptomatic ICA stenosis will reduce microembolic signals measured by TCD and new DWI lesions on magnetic resonance imaging (MRI).

The aim of this randomized controlled trial was to evaluate the safety and efficacy of Revacept in patients with symptomatic ICA stenosis.

METHODS

The study protocol, statistical analysis plan, and anonymized patient data that underlie the results of this article are available upon reasonable request from the corresponding author.

Study Design

The Revacept/CS/02 study (NCT01645306) was an international, prospective, randomized, placebo-controlled, double-blind explorative phase II study with 1:1:1 randomization to one of the 3 treatment groups: placebo, Revacept 40 mg or Revacept 120 mg. The methodological details of the trial are presented in detail elsewhere[11] (see additional material provided by the authors). In brief, adult patients with recently symptomatic, carotid artery stenosis were randomized to either high or low dose Revacept or placebo before they underwent intervention by carotid endarterectomy (CEA)/carotid angioplasty and stenting (CAS) or best medical treatment (BMT). The initial study design included only those patients with detection of MES by screening TCD. Due to the low volume of patients showing MES at screening, on 22.06.2015 (protocol version 8), this inclusion criteria was changed to all patients in whom TCD was possible (adequate bone window) irrespective of the occurrence of MES. Consequently MES was no longer regarded as primary outcome variable.[11]

The study was approved by the UK National Research Ethics Committee East of England (Cambridge South, The Old Chapel, Royal Standard Court, Nottingham, NG16FS) and the lead Ethics Committee in Germany (Ethikkommission der Fakultät für Medizin der Technischen Universität München, Ismaninger Straße 22, 81675 München). The current study is reported according to CONSORT 2010 Statement (Table S1).[12]

Participants

Patients were enrolled in 16 centers in the United Kingdom and Germany from March 8, 2013 until September 27, 2018

(Table S2). Eligible patients were included, if older than 18 years with symptomatic, extracranial ICA stenosis presenting with ischemic stroke, transient ischemic attack, or intermittent blindness (amaurosis fugax) within the last 30 days and at least 50% stenosis of the ICA according to ECST (European Carotid Surgery Trial) duplex criteria.[13] Exclusion criteria included those taking dual antiplatelets, oral anticoagulation, or who had received intravenous thrombolysis within the last 48 hours before screening. Other exclusions were those with concurrent cardiac cause of stroke (eg, atrial fibrillation), recent intracranial hemorrhage, and no acoustic window available for TCD.

Interventions

Patients were identified by the treating clinician and screened by the research team concerning inclusion and exclusion criteria. In case of sufficient bone window for TCD-recordings and written informed consent patients received study medication, manufactured and provided by advanceCOR (Martinsried, Germany), administered by a single intravenous infusion over 20 minutes in 50 mL volume via an infusion pump. TCD-recordings were obtained from the middle cerebral artery with a DWL TCD machine (Compumedics GmbH, Singen, Germany) with a single-depth 2-MHz transducer at screening and after infusion of study medication. Standard settings were used by all study centers.[14] After completion of a sufficient test run, the total number of MES per hour was analyzed by a central core laboratory, led by M. Ritter (Münster, Germany), blinded to clinical data and treatment group. The management of treatment of the symptomatic carotid artery stenosis (CAS, CEA, BMT) was at the discretion of the treating physician. MRI with standardized DWI-sequences for evaluation of new DWI lesions was performed at screening and repeated 24 hours after the revascularization procedure (CAS, CEA) or treatment initiation (BMT). Clinical visits were scheduled 1 day, 3 days, and 3 months after study drug administration (including clinical examination, assessment of National Institutes of Health Stroke Scale (NIHSS), ECG-recording and laboratory assessment). A telephone interview to assess cerebrovascular events was scheduled 12 months after study drug administration.

Randomization and Masking

Eligible subjects were randomized 1:1:1 by the local investigator to one of the 3 treatment groups: placebo, Revacept 40 mg or 120 mg. A computer-generated random block randomization (block size of 3), stratified by: thrombocyte inhibition intake before screening, statin intake before screening and grading of ICA-stenosis was integrated into an independent, secure and validated randomization tool (www.randomizer.at (Medical University of Graz, Institute for Medical Informatics, Statistics and Documentation [IMI]). Treating physicians, patients and study personal assessing outcomes (evaluation of MES and number of DWI-lesions) were blinded to treatment groups.

Efficacy Outcome

The efficacy end point was the number of new lesions on DWI-MRI, per patient. The same scanner acquired images with a standardized protocol at baseline and follow-up (1 day after revascularization procedure). All MRI scans were independently and blindly assessed in a central laboratory by an experienced neuroradiologist (Dr Hauser). New DWI-lesion was defined as increase in signal intensity at least on 2 planes, with corresponding decreased signal intensity detected on apparent diffusion coefficient images. At start of the study period, the reduction of MES assessed by TCD served as main efficacy end point. Due to the low MES-incidence in patients screened for potential study participation, this outcome was dropped according to a protocol change.

Clinical Outcomes

Clinical end points were clinical disability as measured by NIHSS, and predefined clinical efficacy end points, which were considered cumulatively during follow-up including the following cerebrovascular events: any stroke (hemorrhagic or ischemic stroke) or death, transient ischemic attack (transient neurological symptoms, reversible within 24 hours, including amaurosis fugax) or coronary event (myocardial infarction [STEMI or NSTEMI] or necessity of coronary intervention). Moreover, the following safety end points were considered: bleeding complications rated as major bleeding according to the RE-LY (randomized evaluation of long-term anticoagulant therapy) Study[15] (fall in hemoglobin of at least 20 g/liter, transfusion of at least 2 units of blood, symptomatic bleeding in a critical area or organ) or any bleeding—additionally incorporating bleedings rated as adverse event by local investigators. A combined safety and efficacy end point of any cerebrovascular events (ischemic stroke, hemorrhagic stroke, transient ischemic attack, death, myocardial infarction or coronary intervention) or bleeding complications (any bleeding) during the study period served as composite clinical outcome.

Statistical Analysis

The sample size estimation was based on the CARESS study results[5]: assuming a MES incidence of 43.8% (treatment arm) compared with 72.2% (placebo), this study had 80% power to detect a decrease in MES incidence at day 7 after treatment with Revacept (allocation of 50 patients to each treatment group, 2-sided Fisher's exact test, alpha=0.05). Due to low incidence of MES-positive patients and a correspondent protocol change, all statistical tests were performed 2 sided and interpreted in a descriptive, exploratory way. Differences in development of new DWI-lesions on MR-imaging (number of new lesions per patient), as well as number of MES detected by TCD, were analysed using Poisson and, alternatively, negative binomial regression analyses (Tables S3 and S4). Data analysis (further details see Supplemental Methods) was performed by independent data management and biostatistics experts Peter Klein (d.s.h. statistical services GmbH, Rohrbach, Germany) and Christian Rummey (Clinical Data Science GmbH, Basel, Switzerland).

RESULTS

Participants and Baseline Characteristics

From March 8, 2013 until September 27, 2018, 348 patients were screened for eligibility of whom 181 patients were excluded and 7 declined to participate.

After randomization, 2 additional patients were excluded (elevated blood pressure [n=1]) and organizational reasons [n=1]). One hundred fifty-eight patients received study medication and served as patient population for intention-to-treat analysis (ITT, placebo n=51, 40 mg Revacept n=54, 120 mg Revacept n=53, Figure 1). The study population had a mean age of 68 years (SD, 10.1; range, 61–76). Thirty eight of 158 patients (24%) were women; mean duration of follow-up was 11.2±2.3 months. Forty two patients (26.6%) had a 50% to 70% stenosis and 116 patients (73.4%) a stenosis above 70% (ECST-criteria). With regard to management of the symptomatic ICA stenosis, 127 patients (80.4%) underwent CEA, 12 patients (7.6%) were revascularized by CAS, and 19 patients (12.0%) received BMT. The 3 groups were balanced about demographics, clinical, and treatment characteristics (Table), as well as number of MES/h and number of DWI lesions per patient prior study drug administration (Table S3).

MRI at Baseline was available in 139 patients (placebo n=46, Revacept 40 mg n=46, Revacept 120 mg n=47), and follow-up MRI in 126 patients (placebo n=44, Revacept 40 mg n=41, Revacept 120 mg n=41).

Efficacy Outcome

Number of new DWI lesions per patient were 1.16 (95% CI, 0.88–1.53) in the placebo group, 1.05 (95% CI, 0.78–1.42) after treatment with 40 mg Revacept (*P*=0.629) and 0.63 after treatment with 120 mg Revacept ([95% CI, 0.43–0.93]; *P*=0.012; Figure 2A; Table S3). The statistically significant result from the Poisson model was not validated in the negative binomial regression analysis (Tables S4 and S5). Of note, time intervals of the 2 DWI assessments by MRI varied among patients; however, this factor was found to have no statistically significant influence on treatment effects (Table S5). Differences were confirmed in the prespecified subgroup >70% stenosis (Figure 2B) and patients revascularized by CEA (Figure 2C). In accordance, the frequency of patients with new DWI-lesions was equally distributed between treatment groups and in prespecified subgroups (degree of ICA-stenosis, prior thrombocyte inhibition and prior statin treatment) as well as in post hoc analysis (subgroup of patients with MES detected at baseline, management of ICA-stenosis [Figure 3] and concomitant medication with acetylsalicylic acid/clopidogrel/ no thrombocyte inhibition [Table S6]).

Clinical Outcomes

Concerning clinical disability outcome 3 months after study drug administration, the NIHSS showed no difference between the 3 treatment groups (placebo 0 [range 0–1)] Revacept 40 mg 0 [0–1], Revacept 120 mg 0 [0–1], *P*=0.964, Table S7).

With regard to clinical efficacy (cerebrovascular events) and safety end points (bleeding complications) 90 days after study drug administration, no significant differences in distribution between the 3 treatment groups were observed when considering each outcome separately (Table S7). The combined safety and efficacy end point was unchanged 90 days after treatment with 120 and 40 mg Revacept compared with placebo (placebo 16 events [31.4%] versus 120 mg Revacept, 9 events [17.0%], OR, 0.447 [95% CI, 0.177–1.133]; *P*=0.090; placebo versus 40 mg Revacept, 13 events [24.1%], OR 0.694 [95% CI, 0.294–1.639]; *P*=0.404). Time-dependent Cox-regression modeling during the study period revealed a risk reduction for the combined safety and efficacy end point after treatment with 120 mg Revacept compared with placebo (hazard ratio [HR], 0.46 [95% CI, 0.21–0.99]; *P*=0.047), but not 40 mg Revacept (HR, 0.72 [95% CI, 0.37–1.42]; *P*=0.343, Figure 4A). This observation was preserved in the subgroup of patients with >70% stenosis (Figure 4B). When considering safety and efficacy end point separately Revacept showed a nonsignificant trend toward reduction of safety (any bleeding, HR, 0.51 [95% CI, 0.17–1.52]; *P*=0.225) and efficacy events (any stroke, death, transient ischemic attack, myocardial infarction or percutaneous coronary intervention; HR, 0.60 [95% CI, 0.23–1.55]; *P*=0.294; Figure S1).

The effect of predefined subgroups (degree of ICA-stenosis, prior thrombocyte inhibition and prior statin treatment) as well as post hoc analysis (subgroup of patients with MES detected at baseline, management of ICA-stenosis) on the combined safety and efficacy end point showed fewer outcome events after treatment with 120 mg Revacept in the following subgroups: (1) degree of ICA-stenosis above 70%, patients with prior statin therapy, as well as patients undergoing CEA (Figure 5). Contrary, the frequency of patients with occurrence of the combined safety and efficacy end point was equally distributed between treatment groups in prespecified subgroup of patients with prior thrombocyte inhibition, as well as in post hoc analysis of patients with concomitant medication of acetylsalicylic acid/clopidogrel/no thrombocyte inhibition (Table S6) and the subgroup of patients with MES detected at baseline (Figure 5).

Adverse Events

Adverse events were reported for 35 (68.6%), 41 (75.9%), and 32 (60.4%) after treatment with placebo, 40 mg Revacept, and 120 mg Revacept. The distribution of adverse events and serious adverse events showed no significant differences in distribution between the 3 treatment groups (Table S9). An overview of adverse events is given in Table S10 and the incidence of serious adverse events is displayed in Table S11.

Stroke. 2022;53:00–00. DOI: 10.1161/STROKEAHA.121.037006

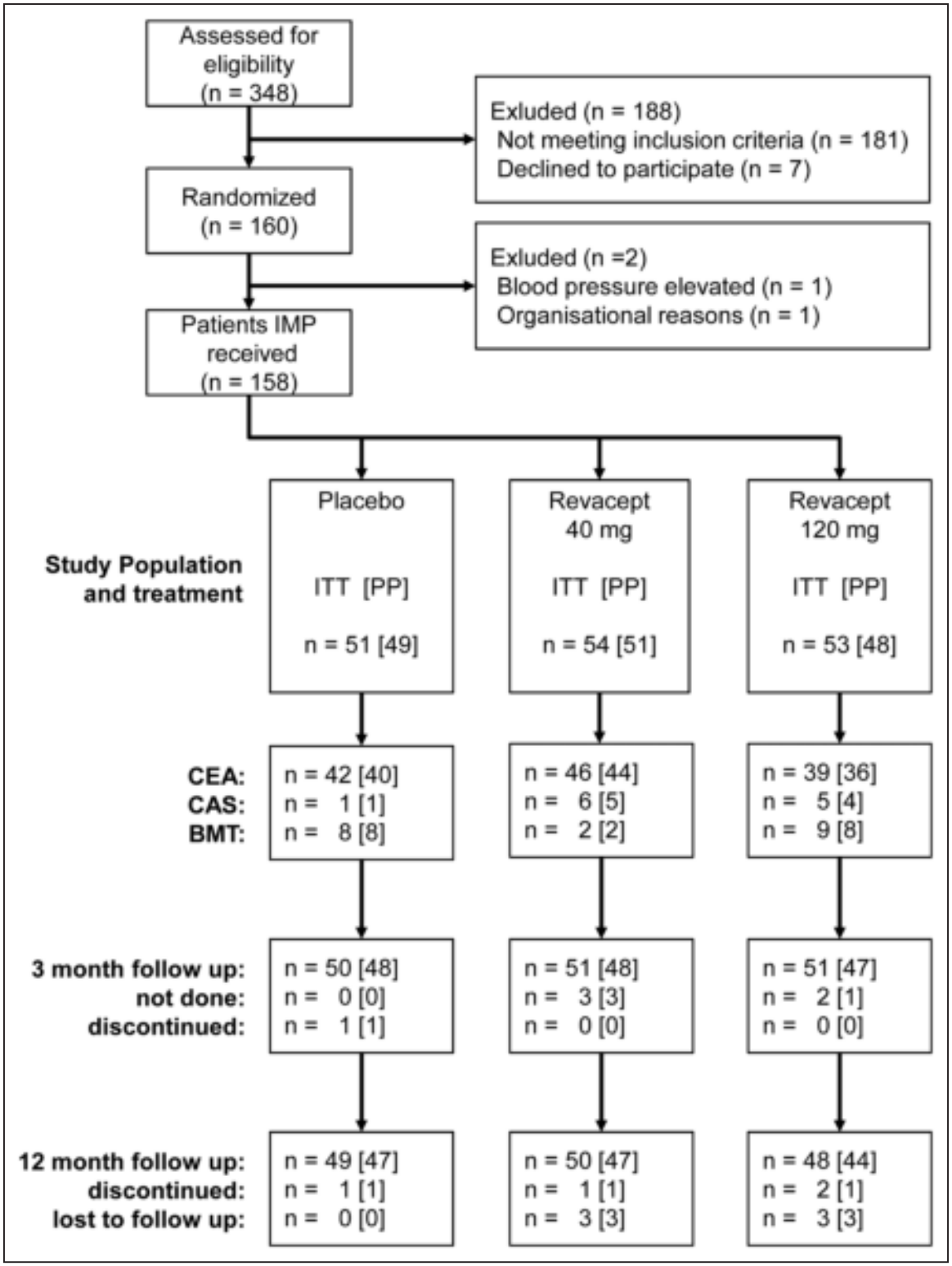

Figure 1. Trial profile–consort diagram.
BMT indicates best medical treatment; CAS, carotid angioplasty and stenting; CEA, carotid endarterectomy; IMP, investigational medical product; ITT, intention to treat population; and PP, per protocol population (displayed in brackets).

DISCUSSION

The Revacept/CS/02 phase II study assessed safety and efficacy of plaque-specific inhibition of platelet activation by the soluble GPVI receptor Revacept in patients with symptomatic ICA stenosis. Revacept, in combination with guideline-recommended antiplatelet therapy, showed a favorable safety profile, arguing for

Table. Baseline Characteristics of the Intention-to-Treat Population

	Placebo	Revacept 40 mg	Revacept 120 mg	*P* value
N	51	54	53	
Mean age, y	67.3 (±10.3)	68.7 (±10.5)	68.4 (±9.8)	0.806
Sex				
Female	8 (15.7)	13 (24.1)	17 (32.1)	0.157
Ethnicity				0.402
African	1 (2.0)	0 (0)	0 (0)	0.351
Asian	1 (2.0)	2 (3.7)	0 (0)	0.379
White	49 (96.1)	52 (96.3)	52 (100)	0.361
Body weight	81.7±18.0	85.5±18.4	83.0±15.4	0.593
BMI	26.9±4.8	29.0±5.1	28.2±5.1	0.077
Medical history				
Arterial hypertension	32 (62.7)	38 (70.4)	35 (66.0)	0.646
Diabetes	12 (23.5)	13 (24.1)	12 (22.6)	0.957
Hyperlipidemia	21 (41.2)	26 (48.2)	15 (28.3)	0.069
Smoking current	17 (33.3)	18 (33.3)	14 (26.4)	0.113
Previous ischemic stroke	5 (9.8)	9 (16.7)	11 (20.8)	0.341
Previous TIA	4 (7.8)	7 (13.0)	7 (13.2)	0.648
Heart failure	1 (2.0)	2 (3.8)	0 (0)	0.361
Coronary artery disease	8 (15.7)	13 (24.1)	8 (15.1)	0.372
Peripheral artery disease	8 (15.7)	6 (11.1)	2 (3.8)	0.110
Blood pressure				
Systolic, mm Hg	138.4±20.4	138.9±17.9	145.3±22.6	0.181
Diastolic, mm Hg	73.2±10.8	74.1±9.8	76.7±12.1	0.235
NIHSS admission	1.0 (0–2)	0 (0–2)	0 (0–1)	0.220
mRS admission	1.0 (0–3.0)	1.0 (0–2.0)	1.0 (0–2.0)	0.285
Concomitant medication				
Antiplatelet	45 (88.2)	47 (87.0)	45 (84.9)	0.879
ASA	40 (78.4)	41 (75.9)	40 (75.5)	0.929
Clopidogrel	5 (9.8)	6 (11.1)	9 (9.4)	0.955
Statins	41 (80.4)	44 (81.5)	45 (84.9)	0.819
LDL, mg/dL	106±36.7	98.2±49.2	122.6±47.4	0.242
Degree of ICA stenosis*				0.986
50%–70%	13 (25.5)	15 (27.8)	14 (26.4)	
>70%	38 (74.5)	39 (72.2)	39 (73.6)	
Revascularization procedure				0.075
CEA	42 (82.4%)	46 (85.2%)	39 (73.6%)	0.291
CAS	1 (2.0%)	6 (11.1%)	5 (9.4%)	0.173
BMT	8 (15.7%)	2 (3.7%)	9 (17.0%)	0.067

Data are n (%), mean±SD, median (interquartile range). ASA indicates acetylsalicylic acid (aspirin); BMI, body mass index; BMT, best medical treatment; CAS, carotid angioplasty and stenting; CEA, carotid endarterectomy; ECST, European Carotid Surgery Trial; ICA, internal carotid artery; LDL, low-density lipoprotein; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; and TIA, transient ischemic attack.

*According to ECST criteria.

a safe administration in patients with an acute cerebrovascular ischemic event. A numerical reduction of new DWI-lesions after the revascularization procedure was observed following treatment with 120 mg Revacept compared with placebo; while the main analyses (Poisson regression) found this difference to be statistically significant, this was not validated by negative binomial regression analysis, so that the result needs to be treated with prudence. In addition, the combined safety and efficacy end point showed a 54% risk reduction during the study period after treatment with the high dose regimen of Revacept (120 mg) compared with placebo, mainly attributable to numerically fewer bleeding complications. The combination of additional protection against thrombi

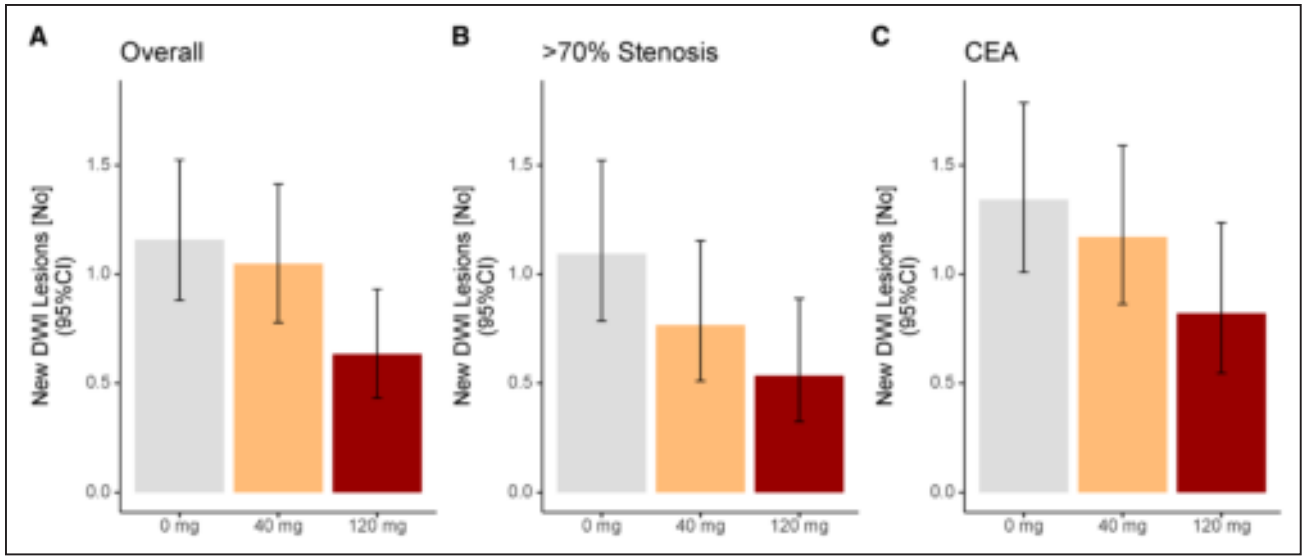

Figure 2. Efficacy end point of new diffusion-weighted magnetic resonance imaging (DWI) lesions.
A, Number of new ischemic lesions per patient on DWI after treatment initiation are numerically reduced after treatment with 40 mg Revacept (orange bar, 1.05 [95% CI, 0.78–1.42]; *P*=0.629) and 120 mg Revacept (red bar, 0.63 [95% CI, 0.43–0.93]; *P*=0.012), compared with placebo (gray bar, 1.16 [95% CI, 0.88–1.53]). **B**, Number of new DWI lesions after treatment initiation in the subgroup of patients with >70% stenosis (ECST-criteria) are reduced from 1.09 (95% CI, 0.78–1.53) in the placebo group (gray bar), to 0.77 (95% CI, 0.51–1.16, *P*=0.186 vs placebo) after 40 mg Revacept (orange bar) and 0.54 (95% CI, 0.32–0.89, *P*=0.021 vs placebo) after 120 mg Revacept (red bar). **C**, Number of new DWI lesions after treatment initiation in the subgroup of patients undergoing carotid endarterectomy (CEA) are reduced from 1.34 (95% CI, 1.01–1.79) in the placebo group (gray bar) to 1.17 (95% CI, 0.86–1.60, *P*=0.349 compared with placebo) after 40 mg Revacept (orange bar) and 0.82 (95% CI, 0.54–1.24, *P*=0.053 compared with placebo) after 120 mg Revacept (red bar).

with no increase in bleedings would be an advantageous feature for platelet inhibition. To date, intensified platelet inhibition carries the risk of increase in bleedings and is a compromise between safety and efficacy.[16]

Patients with recent cerebral ischemia due to extracranial symptomatic ICA stenosis harbor an increased risk for recurrent stroke due to the vulnerable plaque of about 21% within the first 14 days after the initial event.[2,17] In addition, the revascularization procedure itself carries a risk for cardiovascular complications,[18] and silent cerebral ischemic lesions on MRI,[19,20] which are known to deteriorate a patient's clinical outcome. Therefore, patients with symptomatic ICA stenosis might benefit from additional antiplatelet therapy since, as well as during revascularization procedure. With a half-life of around 7 to 14 days, Revacept might represent a promising strategy to bridge the interval between symptom onset and revascularization (CEA, CAS) of ICA-stenosis. Moreover, patients managed by BMT, regularly receiving intensified antiplatelet therapy (eg, combination of acetylsalicylic acid and clopidogrel) might benefit from a site-specific inhibition of platelet activation at the site of the vulnerable plaque by Revacept, without the need of additional compromising general hemostasis.

Unfortunately, we were unable to explore MES as a primary efficacy end point due to the low rate of MES-positive patients at screening. An explanation for the low MES-incidence might be the ameliorated and intensified early secondary preventive therapy between conduction of the Revacept/CS/02 study and the CARESS trial,[5] which was used for the initial treatment protocol. Nevertheless, we were able to evaluate the effect of Revacept on DWI lesions after treatment initiation (CEA, CAS, BMT), which are reported to be associated with the number of MES in patients with symptomatic ICA stenosis and are an established surrogate marker for cerebral embolizations.[21] Although frequently clinically asymptomatic,[19,20] these DWI-lesions are clinically relevant as they are an independent predictor of recurrent stroke[22] and cognitive decline,[23,24] thus deteriorating a patient's clinical outcome. Within the current study, a numerical reduction on the number of new-DWI lesions on MRI performed after the revascularization procedure was observed after treatment with Revacept. The periprocedural exposure of sub endothelial collagen might be a potential source of platelet activation and thereby generating periprocedural ischemic lesions.[19,20] Notably, patients receiving Revacept more frequently underwent CAS (a procedure which is known to be associated with a high incidence of new DWI-lesions) compared to placebo, so that the Revacept groups had a higher probability of procedural- related silent ischemic lesions.[19] Despite this imminent increased risk of periprocedural DWI lesions, patients treated with Revacept showed a dose-dependent numerical reduction of new DWI lesions.

The clinically crucial combined safety and efficacy end point showed a statistically significant reduction after treatment with 120 mg Revacept compared with placebo during the study period. This clinical benefit was even more pronounced in subgroups of patients with

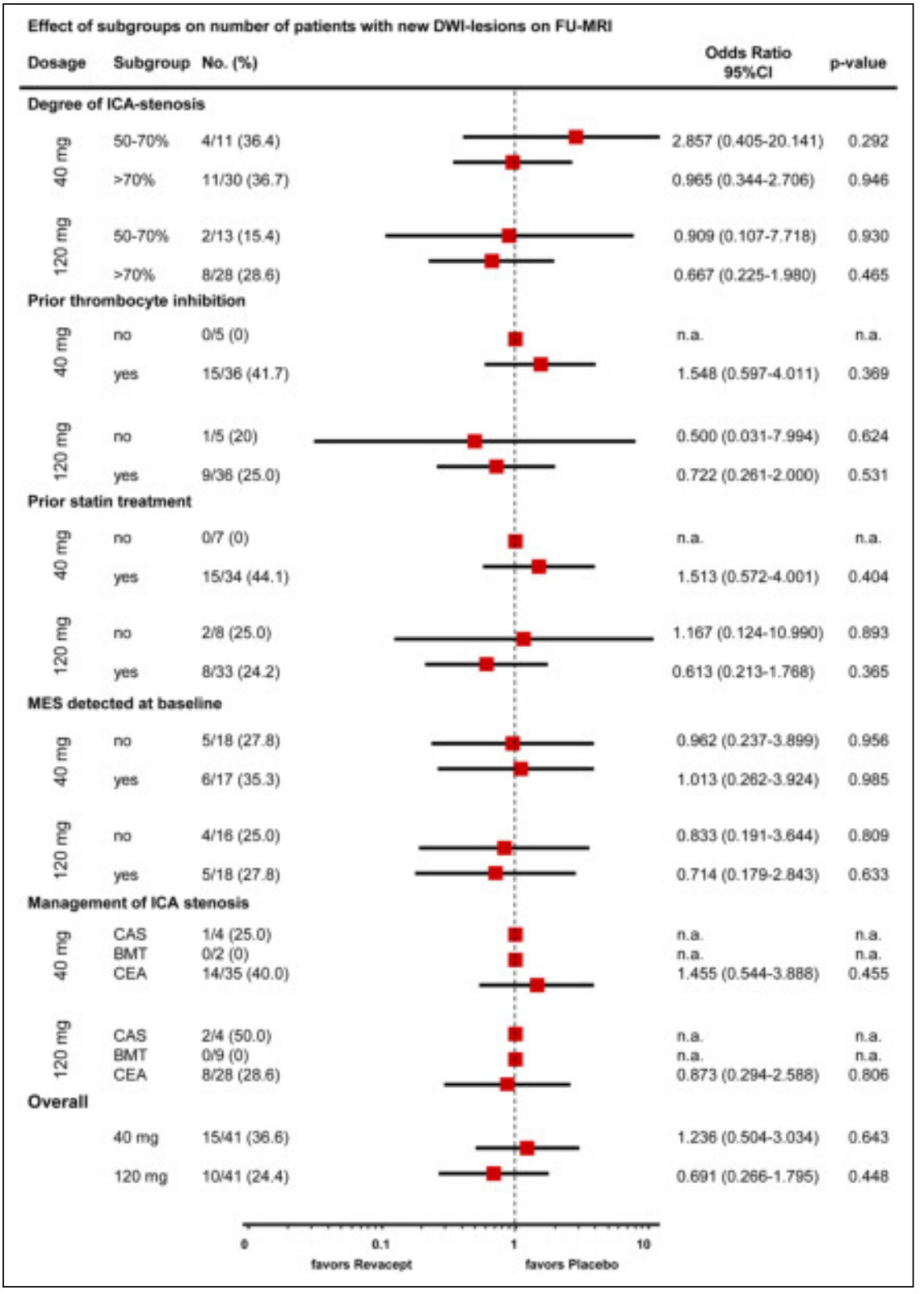

Figure 3. Influence of subgroups on frequency of patients with new diffusion-weighted magnetic resonance imaging (DWI) lesions on magnetic resonance imaging (MRI)–intention to treat analysis.
Predefined subgroups (degree of internal carotid artery [ICA]-stenosis, prior thrombocyte inhibition, prior statin treatment) and posthoc analysis (MES detected at baseline, management of ICA-stenosis) were analyzed by binary logistic regression analysis with prevalence of new DWI-lesions on follow-up MRI as dependent variable and Revacept dosage (40 vs 120 mg.), placebo, as covariate. Displayed is a forest plot with odds ratio (red dot) and 95% CI (black line). Odds ratio values above 1 favor placebo and below 1 favor Revacept treatment.

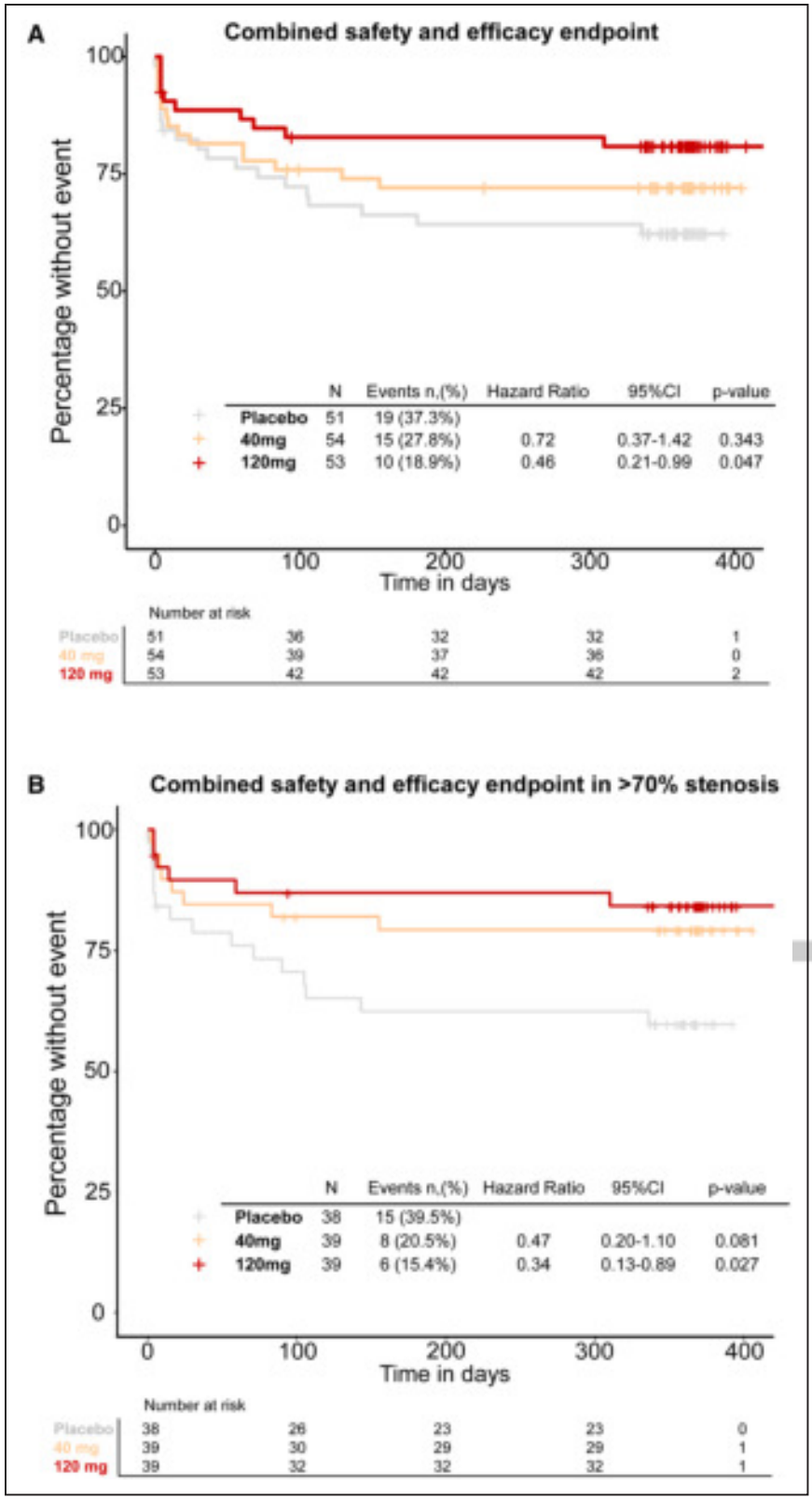

Figure 4. Kaplan-Meier plots for combined safety and efficacy end point–intention to treat analysis. **A**, Time to occurrence of combined safety (any bleeding) and efficacy (any stroke or death, transient ischemic attack (TIA), myocardial infarction, coronary intervention) end point derived from Cox-regression Model. Treatment with Revacept 120 mg (red line) showed a statistically significant reduction in the occurrence of combined safety and efficacy end point compared with placebo (gray line, *P*=0.047). **B**, Time to occurrence of combined safety (any bleeding) and efficacy (any stroke or death, TIA, myocardial infarction, coronary intervention) end point in patients with >70% stenosis (ECST-criteria) of the internal carotid artery. Treatment with Revacept 120 mg (red line) statistically significant reduced the combined safety and efficacy end point compared with placebo (gray line, *P*=0.027). Gray Line (placebo), orange line (40 mg Revacept), red line (120 mg Revacept).

the clinical relevant > 70% stenosis (grading according to ECST-criteria, which corresponds to 50% stenosis according to NASCET (North American Symptomatic Carotid Endarterectomy Trial)-criteria[25]), patients undergoing CEA and patients with previous statin treatment. However, when ischemic events as well as the bleeding complications are analyzed independently, Revacept does not significantly reduce them.

A potential mechanism of action of Revacept might not only be a local antithrombotic effect, but also a prevention

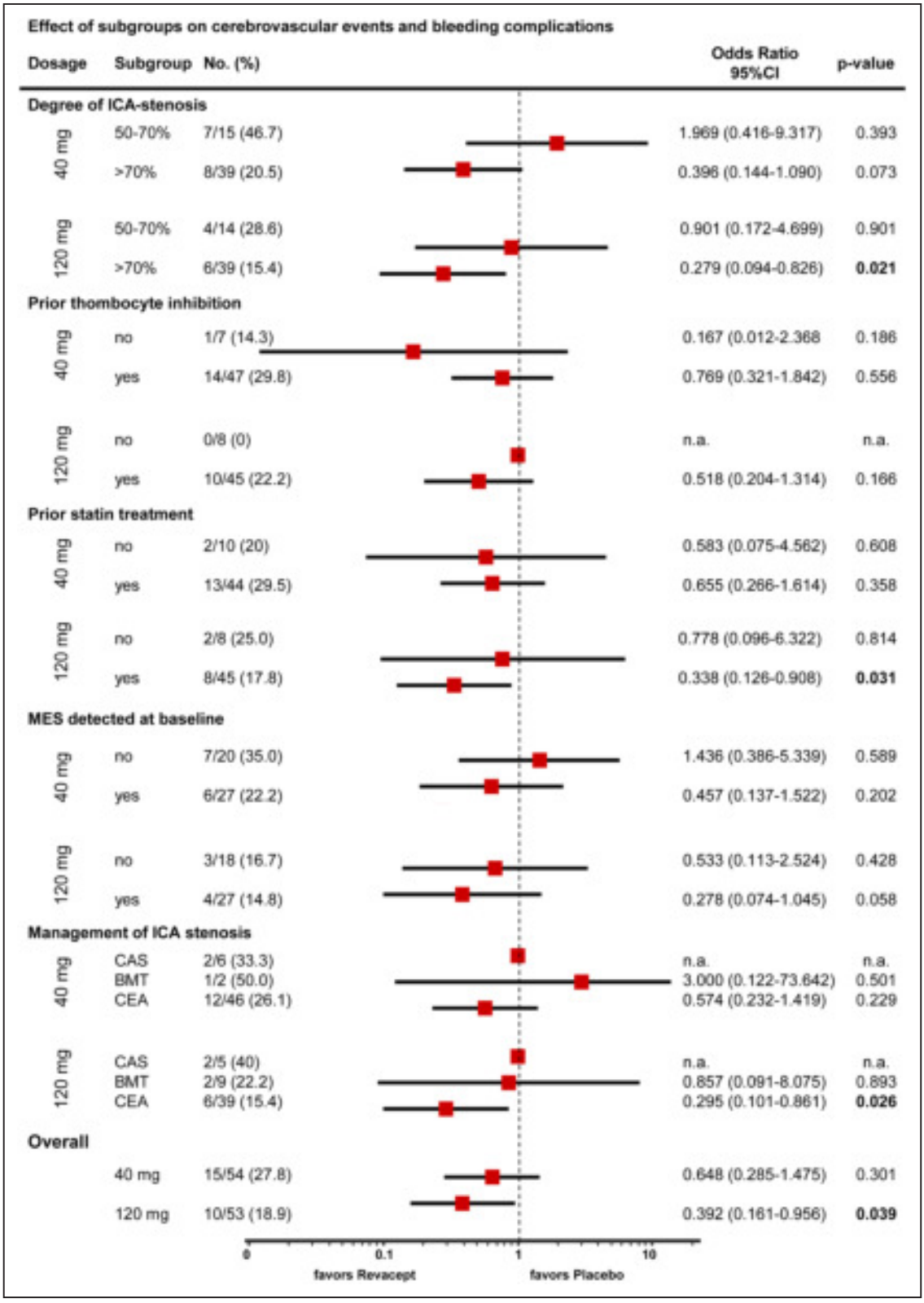

Figure 5. Influence of subgroups on the occurrence of the combined safety and efficacy end point within study period–intention to treat analysis.

Predefined subgroups (degree of internal carotid artery [ICA]-stenosis, prior thrombocyte inhibition, prior statin treatment) and posthoc analysis (microembolic signals detected at baseline, management of ICA-stenosis) were analyzed by binary logistic regression analysis with regard to occurrence of the combined safety and efficacy end point within study period as dependent variable and Revacept dosage (40 vs 120 mg), placebo, as covariate. Displayed is a forest plot with odds ratio (red dot) and 95% CI (black line). OR values above 1 favor placebo and below 1 favor Revacept treatment.

of loss in haemostatic competence possibly by impeding agonist-induced densitization. The phenomenon of platelet dysfunction with reduced collagen-dependent aggregation and defects in haemostatic competence was first described in trauma patients.[26] During active bleeding, the agonist-dependent decrease of GPVI and GPIb (glycoprotein Ib) collagen receptors could also be relevant in vascular patients,[27] so that neutralization of these agonists by Revacept in the atherosclerotic plaque might prevent downregulation of these receptors and thereby support the hemostatic competence. This is underlined by a trend for reduction of bleeding events in the recently published ISAR-PLASTER study (Intracoronary Stenting and Antithrombotic Regimen: Lesion Platelet Adhesion as Selective Target of Endovenous Revacept),[28] examining Revacept in patients with coronary artery disease. However, in contrast to the present study with unstable carotid plaques only, stable coronary artery patients were included in the ISAR-PLASTER study. Procedure-related myocardial infarction mainly caused by side branch obstructions during stenting were not affected by the lesion-specific action of Revacept.

Despite the strengths of the current randomized trial, we are well aware of the potential bias due to the small patient number, different intergroup treatment modalities (CEA, CAS, or BMT) and need for modifying the MES end point during the study, which led to an exploratory analysis of the study results.

CONCLUSIONS

Revacept might represent an option for patients with an acute ischemic stroke due to symptomatic carotid artery stenosis as add-on therapy to guideline-recommended early secondary preventive therapy. The novel mode of action of plaque-specifique inhibition of platelet activation via GPVI and a possible improvement of haemostatic capacity by Revacept paves the way for future phase III studies with underlying ruptured plaque embolization pathologies.

ARTICLE INFORMATION

Received August 17, 2021; final revision received March 18, 2022; accepted April 29, 2022.

Affiliations

Department of Neurology, University Medical Center of the Johannes Gutenberg University Mainz, Germany (T.U., K.G.). Department of Vascular Surgery, University of Western Australia, Fiona Stanley Hospital, Perth, Australia (T.R.). Institute for Medical Informatics, Biometry and Epidemiology, University Hospital, University Duisburg-Essen, Essen, Germany and BDH-clinic Elzach, Germany (C.W.). Department of Neurology, University Hospital Würzburg, Germany (H.N.). Department of Neurology & Stroke, and Hertie Institute for Clinical Brain Research, Eberhard-Karls University Tübingen, Germany (S.P.). Department of Neurology, Hannover Medical School, Germany (K.W.). Warwick Medical School, University of Warwick, Coventry, United Kingdom (C.I.). Department of Neurology, University of Leipzig, Germany (D.M.). Department of Vascular Surgery, King's College Hospital, London, United Kingdom (H.R.). St George's Vascular Institute, St George's Hospital NHS Foundation Trust, London, United Kingdom (I.L.). Clinical Data Science GmbH, Basel, Switzerland (C.R.). Physicians Centre at the Principal Market, Muenster, Germany (M.R.). Department of Diagnostic and Interventional Neuroradiology, University Hospital Tübingen, Germany (T.-K.H.). AdvanceCOR, Martinsried, Germany (G.M.). Department of Neurology, Helios Klinikum München West, Germany (H.P.).

Acknowledgments

We thank Dr Cheryl Ernest for proofreading and editing the article. We acknowledge the contribution of all Revacept participants, investigators, coordinators, and committee members, and staff.

Sources of Funding

This work was supported by advanceCOR, Martinsried, Germany.

Disclosures

Dr Uphaus reports grants from advanceCOR, during the conduct of the study; grants from Else Kröner-Fresenius Stiftung (2018_EKMS.21), personal fees from Merck Serono, personal fees from Pfizer, outside the submitted work. Dr Poli reports grants and personal fees from BMS/Pfizer, grants and personal fees from Daiichi Sankyo, grants from European Union, grants from German Federal Joint Committee Innovation Fund, grants from German Federal Ministry of Education and Research, personal fees from Bayer, grants and personal fees from Boehringer-Ingelheim, personal fees from Portola, personal fees and nonfinancial support from Werfen, personal fees from AstraZeneca, compensation from Alexion Pharmaceuticals, Inc. for consultant services and grants from Helena Laboratories Corporation, outside the submitted work. Dr Loftus reports compensation from Medtronic for consultant services. Dr Hauser reports grants from AdvanceCOR, during the conduct of the study. Dr Münch reports grants from Bundesministerium Bildung und Forschung, personal fees from advanceCOR, during the conduct of the study; other from advanceCOR, outside the submitted work; in addition, Dr Münch has a patent No EP1511770 issued. Dr Gröschel reports grants from advanceCOR, during the conduct of the study; personal fees from Abbott Medical, personal fees from Bayer Vital GmbH, nonfinancial support from Boehringer Ingelheim, personal fees from Bristol-Myers Squibb, nonfinancial support from Daiichi Sankyo, personal fees from Portola, personal fees from Springer Medizin Verlag GmbH, outside the submitted work. Dr Poppert reports grants from advanceCOR, during the conduct of the study; other from Bayer Healthcare paid to his institution for serving on a scientific advisory board, other from Bristol-Myers-Squibb paid to his institution for serving on a scientific advisory board, compensation from Pfizer Pharma GmbH for other services, compensation from Daiichi Sankyo Company for expert witness services, outside the submitted work. The other authors report no conflicts.

Supplemental Material

Supplemental Methods
Tables S1–S11
Figure S1

REFERENCES

1. Kastrup A, Ernemann U, Nägele T, Gröschel K. Risk factors for early recurrent cerebral ischemia before treatment of symptomatic carotid stenosis. *Stroke*. 2006;37:3032–3034. doi: 10.1161/01.STR.0000248968.86868.f3
2. Lovett JK, Coull AJ, Rothwell PM. Early risk of recurrence by subtype of ischemic stroke in population-based incidence studies. *Neurology*. 2004;62:569–573. doi: 10.1212/01.wnl.0000110311.09970.83
3. Valton L, Larrue V, le Traon AP, Massabuau P, Géraud G. Microembolic signals and risk of early recurrence in patients with stroke or transient ischemic attack. *Stroke*. 1998;29:2125–2128. doi: 10.1161/01.str.29.10.2125
4. King A, Markus HS. Doppler embolic signals in cerebrovascular disease and prediction of stroke risk: a systematic review and meta-analysis. *Stroke*. 2009;40:3711–3717. doi: 10.1161/STROKEAHA.109.563056
5. Markus HS, Droste DW, Kaps M, Larrue V, Lees KR, Siebler M, Ringelstein EB. Dual antiplatelet therapy with clopidogrel and aspirin in symptomatic carotid stenosis evaluated using doppler embolic signal detection: the clopidogrel and aspirin for reduction of emboli in symptomatic carotid stenosis (CARESS) trial. *Circulation*. 2005;111:2233–2240. doi: 10.1161/01.CIR.0000163561.90680.1C
6. Almekhlafi MA, Demchuk AM, Mishra S, Bal S, Menon BK, Wiebe S, Clement FM, Wong JH, Hill MD, Goyal M. Malignant emboli on transcranial Doppler during carotid stenting predict postprocedure diffusion-weighted imaging lesions. *Stroke*. 2013;44:1317–1322. doi: 10.1161/STROKEAHA.111.000659

7. Shahidi S, Owen-Falkenberg A, Gottschalksen B, Ellemann K. Risk of early recurrent stroke in symptomatic carotid stenosis after best medical therapy and before endarterectomy. *Int J Stroke*. 2016;11:41–51. doi: 10.1177/1747493015609777
8. Abou-Chebl A, Yadav JS, Reginelli JP, Bajzer C, Bhatt D, Krieger DW. Intracranial hemorrhage and hyperperfusion syndrome following carotid artery stenting: risk factors, prevention, and treatment. *J Am Coll Cardiol*. 2004;43:1596–1601. doi: 10.1016/j.jacc.2003.12.039
9. Jamasbi J, Megens RT, Bianchini M, Münch G, Ungerer M, Faussner A, Sherman S, Walker A, Goyal P, Jung S, et al. Differential inhibition of human atherosclerotic plaque-induced platelet activation by dimeric GPVI-Fc and Anti-GPVI antibodies: functional and imaging studies. *J Am Coll Cardiol*. 2015;65:2404–2415. doi: 10.1016/j.jacc.2015.03.573
10. Ungerer M, Rosport K, Bültmann A, Piechatzek R, Uhland K, Schlieper P, Gawaz M, Münch G. Novel antiplatelet drug revacept (Dimeric Glycoprotein VI-Fc) specifically and efficiently inhibited collagen-induced platelet aggregation without affecting general hemostasis in humans. *Circulation*. 2011;123:1891–1899. doi: 10.1161/CIRCULATIONAHA.110.980623
11. Gröschel K, Uphaus T, Loftus I, Poppert H, Diener HC, Zobel J, Münch G. Revacept, an inhibitor of platelet adhesion in symptomatic carotid artery stenosis: design and rationale of a randomized phase II clinical trial. *TH Open*. 2020;4:e393–e399. doi: 10.1055/s-0040-1721078
12. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *PLoS Med*. 2010;7:e1000251. doi: 10.1371/journal.pmed.1000251
13. European Carotid Surgery Trialists' Collaborative Group. Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the mrc european carotid surgery trial (ECST). *Lancet*. 1998;351:1379–1387. doi: https://doi.org/10.1016/S0140-6736(97)09292-1
14. Ringelstein EB, Droste DW, Babikian VL, Evans DH, Grosset DG, Kaps M, Markus HS, Russell D, Siebler M. Consensus on microembolus detection by TCD. International consensus group on microembolus detection. *Stroke*. 1998;29:725–729. doi: 10.1161/01.str.29.3.725
15. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, et al; RE-LY Steering Committee and Investigators. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2009;361:1139–1151. doi: 10.1056/NEJMoa0905561
16. McFadyen JD, Schaff M, Peter K. Current and future antiplatelet therapies: emphasis on preserving haemostasis. *Nat Rev Cardiol*. 2018;15:181–191. doi: 10.1038/nrcardio.2017.206
17. Fairhead JF, Mehta Z, Rothwell PM. Population-based study of delays in carotid imaging and surgery and the risk of recurrent stroke. *Neurology*. 2005;65:371–375. doi: 10.1212/01.wnl.0000170368.82460.b4
18. Moresoli P, Habib B, Reynier P, Secrest MH, Eisenberg MJ, Filion KB. Carotid stenting versus endarterectomy for asymptomatic carotid artery stenosis: a systematic review and meta-analysis. *Stroke*. 2017;48:2150–2157. doi: 10.1161/STROKEAHA.117.016824
19. Bonati LH, Jongen LM, Haller S, Flach HZ, Dobson J, Nederkoorn PJ, Macdonald S, Gaines PA, Waaijer A, Waajier A, et al; ICSS-MRI study group. New ischaemic brain lesions on MRI after stenting or endarterectomy for symptomatic carotid stenosis: a substudy of the International Carotid Stenting Study (ICSS). *Lancet Neurol*. 2010;9:353–362. doi: 10.1016/S1474-4422(10)70057-0
20. Schnaudigel S, Gröschel K, Pilgram SM, Kastrup A. New brain lesions after carotid stenting versus carotid endarterectomy: a systematic review of the literature. *Stroke*. 2008;39:1911–1919. doi: 10.1161/STROKEAHA.107.500603
21. Kandiyil N, MacSweeney ST, Heptinstall S, May J, Fox SC, Auer DP. Circulating microparticles in patients with symptomatic carotid disease are related to embolic plaque activity and recent cerebral ischaemia. *Cerebrovasc Dis Extra*. 2019;9:9–18. doi: 10.1159/000495942
22. Gensicke H, van der Worp HB, Nederkoorn PJ, Macdonald S, Gaines PA, van der Lugt A, Mali WPTM, Lyrer PA, Peters N, Featherstone RL, et al. Ischemic brain lesions after carotid artery stenting increase future cerebrovascular risk. *J Am Coll Cardiol*. 2015;65:521–529. doi: 10.1016/j.jacc.2014.11.038.
23. Capoccia L, Speziale F, Gazzetti M, Mariani P, Rizzo A, Mansour W, Sbarigia E, Fiorani P. Comparative study on carotid revascularization (endarterectomy vs stenting) using markers of cellular brain injury, neuropsychometric tests, and diffusion-weighted magnetic resonance imaging. *J Vasc Surg*. 2010;51:584–591, 591.e1. doi: 10.1016/j.jvs.2009.10.079
24. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and the risk of dementia and cognitive decline. *N Engl J Med*. 2003;348:1215–1222. doi: 10.1056/NEJMoa022066
25. Arning C, Widder B, von Reutern GM, Stiegler H, Görtler M. [Revision of DEGUM ultrasound criteria for grading internal carotid artery stenoses and transfer to NASCET measurement]. *Ultraschall Med*. 2010;31:251–257. doi: 10.1055/s-0029-1245336
26. Vulliamy P, Montague SJ, Gillespie S, Chan MV, Coupland LA, Andrews RK, Warner TD, Gardiner EE, Brohi K, Armstrong PC. Loss of GPVI and GPIbα contributes to trauma-induced platelet dysfunction in severely injured patients. *Blood Adv*. 2020;4:2623–2630. doi: 10.1182/bloodadvances.2020001776
27. Gardiner EE, Arthur JF, Kahn ML, Berndt MC, Andrews RK. Regulation of platelet membrane levels of glycoprotein VI by a platelet-derived metalloproteinase. *Blood*. 2004;104:3611–3617. doi: 10.1182/blood-2004-04-1549
28. Mayer K, Hein-Rothweiler R, Schüpke S, Janisch M, Bernlochner I, Ndrepepa G, Sibbing D, Gori T, Borst O, Holdenrieder S, et al. Efficacy and safety of revacept, a novel lesion-directed competitive antagonist to platelet glycoprotein VI, in patients undergoing elective percutaneous coronary intervention for stable ischemic heart disease: the randomized, double-blind, placebo-controlled ISAR-PLASTER phase 2 trial. *JAMA Cardiol*. 2021;6:753–761. doi: 10.1001/jamacardio.2021.0475